J. Helminthol. Soc. Wash.
66(1), 1999 pp. 73–77

Research Note

Helminths Infecting Froglets of the Northern Leopard Frog (*Rana pipiens*) from Foggy Bottom Marsh, Michigan

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ABSTRACT: Twelve helminth taxa (6 Nematoda, 5 Trematoda, and 1 Cestoda) were found in 43 froglets of the northern leopard frog, *Rana pipiens* Schreber, from Foggy Bottom Marsh in southern Michigan during July–October 1997. The cestode, *Mesocestoides* sp., had the highest mean intensity. Four taxa of larval trematodes occurred in the froglets, with the strigeid metacercariae having the highest prevalence and mean abundance and the unidentified metacercariae having the highest mean intensity. *Clinostomum* sp. and *Fibricola* sp. were also found. Of the nematodes, *Oswaldocruzia priceae* had the highest prevalence, and *Rhabdias ranae* had the highest mean intensity and mean abundance. Larval digeneans were the first members of the helminth community to become established in the froglets, along with *O. priceae* and *Cosmocercoides dukae*. Taxonomically, 536 (40%) cestodes, 484 (36%) trematodes, and 309 (23%) nematodes were found in the froglets. Although there are reports of parasites causing amphibian deformities, abnormalities were not observed in froglets that were commonly infected with larval helminths. One adult leopard frog was infected with *Mesocestoides* sp., *O. priceae*, *R. ranae*, and *Gorgodera amplicava*.

KEY WORDS: *Rana pipiens*, northern leopard frog, froglet, helminths, Nematoda, Trematoda, Cestoda, deformities, Michigan.

Parasites of the northern leopard frog in North America have been investigated by several authors: Faust (1918), Hegner (1922), Walton (1929), Reiber et al. (1940), Chandler (1942), Ulmer (1970), Baker (1976, 1978, 1985), Levine and Nye (1977), Williams and Taft (1980), Martin and Conn (1990), McAllister and Conn (1990), and McAlpine (1997). Jewell (1916), Cort (1917), Fortner (1923), Cort and Brooks (1928), Hughes (1928), Krull (1930, 1931), Talbot (1933), Cort and Brackett (1938), Olivier and Odlaug (1938), Herber (1939), Lawler (1939), Thomas (1939), Olivier (1940, 1942), Najarian (1952, 1953a, b, 1954, 1955), Ridgeway (1964), and Werner and Walewski (1976) all reported on the parasites of Michigan leopard frogs. As far as we know, McAlpine's (1997) is the only publication on parasites of young and juveniles of this species in North America. Populations of anurans, including leopard frogs, with limb abnormalities occur throughout North America. Sessions and Ruth (1990) found metacercariae in close association with hindlimb deformities of the Pacific tree frog, *Hyla regilla*, and the long-toed salamander, *Ambystoma macrodactylum*. They suggested that metacercariae cause a mechanical disruption in limb growth, thus explaining the presence of limb deformities. The objectives of our study were to survey the helminths of froglets in a natural population of northern leopard frogs and to report if metacercariae were associated with limb abnormalities.

A total of 44 frogs (43 froglets and 1 adult) were collected by hand or dip net during the day in July–October 1997 from Foggy Bottom Marsh, Mason County, southern Michigan. It has an area of approximately 40 acres and ranges in depth from 0.5 to 1 m. Cattails (*Typha* spp.) and various sedges border the marsh's edge, and oaks (*Quercus* spp.) and maples (*Acer* spp.) surround the northern and eastern shores. We consider a froglet to be a young of the year individual with both forelimbs and hindlimbs and not of breeding size. Froglets less than 5 mo old were collected on 5 dates, 2 wk apart, mostly in the transition area between land and water and in water approximately 20 cm in depth. Frogs were pithed, and most were examined within 48 hr; a few were fixed in 20% formalin and preserved in 70% EtOH. The mean snout–vent length (mm ± 1 SD) of the 43 froglets was 43.3 ± 6.8, range = 30–57 mm. The lungs, liver, gall bladder, stomach, small intestine, rectum, urinary bladder, abdominal cavity, mesentery, and musculature were examined. The helminths that were collected were processed using conven-

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Table 1. Prevalence, mean intensity, mean abundance, and total number of helminths occurring in 43 froglets of the northern leopard frog from Foggy Bottom Marsh, Michigan, during July–October 1997.

	Prevalence	Mean intensity ±1 SD (max.)	Mean abundance ±1 SD	No. of helminths found (% of community)	Location in host†
Digenea					
Clinostomum sp.*	19	2.5 ± 2.3 (7)	0.46 ± 1.4	20 (1.5)	US, LM
Fibricola sp.*	5	5.0 ± 2.8 (7)	0.23 ± 1.2	10 (0.75)	LF
Immature plagiorchid	2	1.0	0.02 ± 0.15	1 (0.08)	R
Strigeid metacercariae*	49	22.3 ± 43.4 (160)‡	9.71 ± 30.3‡	378 (28.4)‡	LM, EH, US, ESI, ME, LV, BC
Unidentified metacercariae*	7	25.0 ± 23.0 (50)	1.7 ± 8.2	75 (5.6)	K
Cestoda					
Mesocestoides sp.*	9	134.0 ± 153.1 (283)	12.3 ± 56.8	536 (40.3)	ME, ESI, EST, LV, LF, US, BC
Nematoda					
Cosmocercoides dukae	19	2.1 ± 1.8 (6)	0.4 ± 0.1	17 (1.2)	SI, R
Oswaldocruzia priceae	33	8.8 ± 12.6 (50)	2.9 ± 8.1	123 (9.2)	SI
Raillientnema sp.	7	7.6 ± 9.8 (19)	0.5 ± 2.9	23 (1.7)	SI, R
Rhabdias ranae	26	13.0 ± 15.8 (40)	3.3 ± 9.6	143 (10.7)	L
Spiroxys sp.*	5	1.0	0.05 ± 0.21	2 (0.15)	EST, ESI
Immature larva*	2	1.0	0.02 ± 0.15	1 (0.08)	LM

* Larval stage.

† Location in host: US = under skin, LM = encysted in leg muscle, LF = free in leg muscle, R = rectum, EH = external surface of heart, ESI = external surface of small intestine, ME = mesentery, K = kidneys, BC = body cavity, LV = liver, EST = external surface of stomach, SI = small intestine, L = lungs.

‡ Values based on 17 infected froglets.

tional techniques. Prevalence is the percentage of froglets infected, mean intensity is the mean number of parasites per infected froglet, and mean abundance is the mean number of parasites per examined froglet. Strigeid metacercariae were not counted in 4 froglets; therefore, these strigeid numbers were not included in the calculations for mean intensity, mean abundance, and total mean helminth abundance. Values are expressed as a mean ± 1 SD. Voucher specimens have been deposited in the United States National Parasite Collection, Beltsville, Maryland: *Gorgodera amplicava* (No. 88222), *Cosmocercoides dukae* (No. 88247), *Oswaldocruzia priceae* (No. 88223), and *Rhabdias ranae* (No. 88224).

Twelve helminth taxa infected froglets of the northern leopard frog, including 4 larval trematode taxa (Table 1). The *Clinostomum* sp. was easily seen in a large cyst. The diplostomulum type metacercariae of *Fibricola* sp. was found free or enveloped by host tissue and was identified by having oral and ventral suckers, the absence of lateral pseudosuckers, a holdfast organ with a slitlike opening posterior to the ventral sucker, intestinal ceca enlarged, tegument with spines, and a small conical hindbody. Strigeid metacercariae were small and were identified by having oral and ventral suckers, a holdfast organ posterior to the ventral sucker, intestinal ceca not enlarged, and faintly seen spines. Over 150 strigeid metacercariae were counted in the hindlimb of 1 froglet. The unidentified metacercariae had oral and ventral suckers, lacked a collar of spines around the oral sucker, and were found only in the kidneys.

Larval cestode tetrathyridia had the highest mean intensity and mean abundance. These were identified by possessing a deeply invaginated and inverted unarmed scolex with 4 suckers, and the high tetrathyridia intensity in the present study is suggestive of *Mesocestoides* sp. More than 160 tetrathyridia were reported by McAllister and Conn (1990) from 1 leopard frog in New York. Four nematode genera, including immature *R. ranae,* occurred in the digestive tract. Of the helminths identified to genus, *O. priceae* had the highest prevalence. Our specimens of *O. priceae* were identified using the key in Ben Slimane and Durette-Desset (1997). The single adult leopard frog (snout–vent length = 76 mm) collected in September harbored 221 *Mesoces-*

Table 2. Summary of the parasites of *Rana pipiens* and their prevalences from Michigan.

Parasite	% Prevalence	Reference
Protozoa		
Nyctotherus cordiformis	2 (1917), 22 (1919)†	Fortner (1923)
Octomitus intestinalis	30 (1917), 48 (1919)	Fortner (1923)
Opalinia obtrigonoidea	61 (1917), 80 (1919)	Fortner (1923)
Trypanasoma pipientis	8	Werner and Walewski (1976)
Trypanasoma ranarum	17	Werner and Walewski (1976)
Trypanasoma rotatorium	71	Werner and Walewski (1976)
Digenea		
*Alaria mustelae**	‡	Olivier and Odlaug (1938)
*Alaria marcianae**	‡	Cort (1917)
	‡	Cort and Brooks (1928)
*Apharyngostrigea pipientis**	‡	Hughes (1928)
Cephalogonimus americanus	20	Najarian (1955)
*Cercaria elodes**	‡	Olivier (1942)
*Clinostomum attenuatum**	2 (1917)	Fortner (1923)
Clinostomum sp.*	19§	This paper
*Diplostomum micradenum**	‡	Olivier (1940)
	‡	Cort and Brackett (1938)
*Echinoparyphium flexum**	42	Najarian (1952)
	42	Najarian (1953a)
	‡	Najarian (1953b)
	50	Najarian (1954)
Fibricola sp.*	5§	This paper
Gorgodera amplicava	100‖	This paper
Gorgodera attenuata	51 (1917), 38 (1919)	Fortner (1923)
Haematoloechus medioplexus	‡	Krull (1930)
	‡	Krull (1931)
	5 (1917), 30 (1919)	Fortner (1923)
Haematoloechus similiplexus	1 (1917), 0.9 (1919)	Fortner (1923)
Halipegus occidualis	<1	Thomas (1939)
*Lechriorchis primus**	‡	Talbot (1933)
Megalodiscus temperatus	30 (1917), 1 (1919)	Fortner (1923)
	63	Herber (1939)
Immature plagiorchid	2§	This paper
Renifer sp.*	20	Najarian (1955)
Strigeid metacercariae*	49§	This paper
Unidentified metacercariae*	7§	This paper
Cestoda		
Cylindrotaenia americana	‡	Jewell (1916)
	‡	Lawler (1939)
Mesocestoides sp.*	9§	This paper
Proteocephalidae	1 (1919)	Fortner (1923)
Nematoda		
Cosmocercoides dukae	19§	This paper
Oswaldocruzia priceae	33§	This paper
Oswaldocruzia sp.	‡	Ridgeway (1964)
Raillientnema sp.	7§	This paper
Rhabdias ranae	26§	This paper
Spiroxys sp.*	5§	This paper
Immature larva*	2§	This paper

* Larval stage.
† Prevalence (year of study).
‡ Present but prevalence not indicated.
§ Prevalence calculated from 43 froglets.
‖ Found in 1 adult.

toides sp. (encysted in the liver, external surface of the stomach and small intestine, and free in the body cavity), 8 *O. priceae* (small intestine), 4 *R. ranae* (lungs), and 3 *G. amplicava* (urinary bladder).

Of the 38 (88%) froglets infected with ≥1 helminths, 18 harbored 1 taxon, 7 harbored 2 taxa, 7 harbored 3 taxa, 5 harbored 4 taxa, and 1 harbored 6 taxa. The mean helminth species richness for all froglets was 1.8 ± 1.4 (range = 0–6). A significant correlation existed between the number of helminth species and froglet length (Spearman's correlation, $r_s = 0.69$, $P < 0.01$). Although not significant, helminth richness values increased with each subsequent collection; they were (range, number of froglets examined): for July 24, 0.6 ± 0.51 (0–1, 10); August 6, 1.5 ± 1.1 (0–4, 11); August 23, 1.7 ± 0.8 (0–3, 11); September 17, 3.2 ± 1.6 (0–6, 9); and October 8, 3.5 ± 0.7 (0–4, 2). The total mean helminth abundances ± SE for the 34 infected froglets and for the 34 infected and 5 uninfected froglets were 39.1 ± 11.9 and 34.0 ± 10.6, respectively. These values are higher than the total mean helminth abundances in young (15.3 ± 3.9) and juvenile (12.7 ± 2.5) leopard frogs reported by McAlpine (1997).

By major parasite group, 536 (40%) cestodes, 483 (36%) trematodes, and 309 (23%) nematodes were recovered from the froglets. In our samples, larval digeneans were the first members of the helminth community to become established in froglets, along with *O. priceae* and *Cosmocercoides dukae*. Likewise, McAlpine (1997) reported larval *Echinostoma trivolvis* and *Apharyngostrigea pipientis* to first infect young leopard frogs. *Mesocestoides* sp. was the last helminth species to be recruited by froglets in the present study.

Twenty-three studies including the present one have investigated some aspect of the parasites of Michigan leopard frogs. However, the only study on parasites from the Upper Peninsula is that of Werner and Walewski (1976), who investigated blood parasites. Ridgeway (1964) provided the most recent report on a helminth of Michigan leopard frogs. A total of 37 parasite taxa (6 Protozoa, 21 Trematoda, 3 Cestoda, and 7 Nematoda) have been found in leopard frogs from Michigan (Table 2). Thirteen larval trematode taxa have been reported from Michigan leopard frogs. The literature reports concerning larval trematode taxonomy, systematics, specific sites found within this anuran species, measurements of larvae, and observable morphological variation are often confusing. Of the taxa identified in the present study, *Clinostomum* sp. and *Oswaldocruzia* sp. are the only parasites to have been previously reported in this frog species in Michigan. The other 10 helminth taxa represent new host records in Michigan.

Sessions and Ruth (1990) reported that metacercariae found in close proximity to deformed limbs caused the abnormalities seen in *H. regilla* and *A. macrodactylum*. They did not specify, however, the number of metacercariae present in the leg musculature of deformed amphibians. Sessions and Ruth (1990) concluded that the timing of infection was important for the development of abnormalities, which could explain why some infected amphibians lacked deformities. These authors implanted inert resin beads in the developing hindlimb buds of laboratory-bred frogs and salamanders and produced the abnormalities seen in wild-caught amphibians. If the presence of metacercariae could cause a mechanical disruption in limb development as suggested by Sessions and Ruth (1990), might it be possible, if their hypothesis is correct, that other larval helminths found within the musculature of the hindlimbs could cause deformities? However, although larval helminths commonly occurred within the muscles of the hindlimbs of the northern leopard frog in the present study, no limb abnormalities were seen in froglets.

We thank W. E. Cooper, Michigan State University, for allowing access to Foggy Bottom Marsh and for his assistance in collecting frogs; C. R. Peebles, Michigan State University, for help in nematode identification and review of the manuscript; D. B. Conn, Berry College, for sharing his thoughts on the identification of *Mesocestoides* tetrathyridia; A. D. Hernandez, K. A. Lehnert, G. M. Utter, and D. S. Hulefeld for help in collecting; and an anonymous reviewer, who provided literature on the taxonomy of *Oswaldocruzia* spp.

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